Arizona Citrus Research Council
Research Report 2003-2004

Project Title: Evaluation of exotic and native strains of entomopathogenic nematodes for the control of the citrus nematode, *Tylenchulus semipenetrans*

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In Arizona, the citrus nematode, *Tylenchulus semipenetrans* is considered the main plant-parasitic nematode species of citrus. Ninety percent of the citrus in the state has been reported to be affected by this nematode. At present, Arizona's citrus growers have few alternatives for controlling of the citrus nematode. According to published reports, application of currently available nematicides has not provided adequate control of the citrus nematode in the desert-grown citrus that are flood-irrigated. On the other hand, aldicarb, although successful in controlling citrus nematode, has not been favored in Arizona due to this high toxicity to mammals, mobility in soil water and the need for mechanical application into the soil. In addition none of the nematode-resistant rootstocks adopted in California and Florida have been found suitable for Arizona's climatic conditions. Consideration of a biological control alternative for the citrus nematode was investigated by Nigh et al. (1997-Citrus Res. Report, pp. 82-90). The application of the insecticidal nematode (also known as entomopathogenic nematode) *Steinernema riobrave* was considered for both the control of citrus nematode and *Liohippelates* eye gnat. In their final report, these authors concluded that because of inappropriate procedures prior to the application of EPN (no evaluation of nematode viability after dilution of commercial product was done) negative results in all trials were obtained. These negative results should not discourage the use of EPN for control of the citrus nematode. On the contrary, this clearly points out that further studies, particularly at the laboratory or greenhouse level, are needed to fully assess the interactions and control potential of these nematodes against the citrus nematode prior to any field-scale trials.

With this background, in this project we considered: the screening of the entomopathogenic nematode species *S. riobrave* to determine the manner in which this entomopathogenic parasite affects the infectivity and reproduction of the citrus nematode. Two concentration rates (high and low) and two different application times (simultaneous and after *T. semipenetrans* establishment) were evaluated to assess the effect of this nematode on *T. semipenetrans*.

Growth of citrus seedlings

Rough lemon seedlings were obtained from J. Loghry (Yuma Ag. Center). The seedlings were about 10 in tall when they were received and they were planted in sterilized sandy soil mixed in 1 pint pots. These seedlings were allowed to established and grow in the greenhouse for about two months before they were inoculated with the citrus nematode. Greenhouse temperature was maintained at 75 ºF

Because of difficulties in citrus nematode establishment into these plants (presumably because were too fibrous), we decided to grow our own seedlings. For this, we followed procedures described by El Borai et al. (2003). Briefly, seeds were surface-sterilized with 10% bleach containing 0.01% Tween-20 for 10 min and then rinsed 5 min in sterile distilled water. One single decorticated seed was placed in a 2cm (1 inch) depression made in the center of the surface of the soil in Ray-Leach cone-tainers™. Seedlings were allowed to germinate and grow for approximately 2 months in a growth chamber (26 ºC ± 2) with continuous white fluorescent light, before they were inoculated with the citrus nematode.

Nematode culture and establishment

Tylenchulus semipenetrans was isolated from citrus nematode-infested soil from three orchards in Yuma. Two different approaches were considered to improve nematode extraction from the infested roots. In the first approach, we considered cutting the roots into 1-cm pieces and placed them in funnels in a mist chamber for approximately 2-3 days at 24 ºC. Because very few juveniles (J2) were recovered with this method, we decided to follow extraction procedures described by Tarjan (1967). Briefly this method considers soaking the roots (cut into 1 cm pieces) in a 3% hydrogen peroxide solution inside Ziplock™ type plastic bags for 12-24 h. After this period, the roots were rinsed three times in tap water and then placed overnight into funnels in the mist chamber. This last method allowed a very high recovery of nematodes, approximately 10-20 times more than with the previous method.

Extracted nematodes were propagated and maintained in culture in lemon seedlings that were allowed to grow for 2 months prior to their inoculation. Approximately 10,000 citrus nematodes (a mixture of J2 (second stage juveniles) and preadult stages) were inoculated into each cone-tainer. Nematodes were allowed to established (i.e. infect the roots and reproduced) for approximately 6-8 wks.

***Steinernema riobrave* culture and inoculation procedures**

Because of difficulties in the establishment of the citrus nematode in the rough lemon seedlings (see comments in above sections) we were only able to consider one entomopathogenic nematode species, *Steinernema riobrave,* for these experiments the TX strain, originally recovered in Texas was considered.

Two inoculum concentrations and two inoculation periods were considered: a) *S. riobrave* was applied 2 months after inoculation of seedlings with the citrus nematodes and b) *S. riobrave* was simultaneously applied with the citrus nematode into two-month old rough lemon seedlings. The following parameters were analyzed:

- Efficacy of *S. riobrave* concentrations on citrus nematode penetration: Two concentrations 100 (low) and 1,000 (high) of *S. riobrave* infective juveniles were considered. Concentration of *S. riobrave* inoculum was based on available information for field application of these nematodes. Generally, a low dosis approximately considers 250 nematodes/plant and the high dosis is of approximately 2,500 nematodes/cm ^{2}. Since

the diameter of the cone-tainers is 4 cm^2, the nematode concentrations were adjusted accordingly.

Efficacy of *S. riobrave* application time on citrus nematode penetration: Both high and low inoculum doses were considered in these experiments. *S. riobrave* infective-juveniles (IJs) were applied at two different times: a) two-moths after establishment of the citrus nematode and b) simultaneous application (i.e. both citrus nematodes and *S. riobrave* applied at the same time). In both situations nematodes were allowed to "interact" with each other for a two-month period. After this, seedlings were removed and the roots were cut and weight (wet weight). Roots were posteriorly stained with fuchsin acid to assess nematode penetration and count for egg masses. Soil from each cone-tainer was placed in the mist extraction chamber for recovery of juveniles and males of *T. semipenetrans.*

There were 10 plants per inoculation time and per inoculum. Controls consisted of 10 plants inoculated only with the citrus nematode and 10 non-inoculated (nematode free) plants. All experiments were repeated twice and statistical analysis will be performed using SAS statistical software package (SAS, Cary, NC)

Results

In the simultaneous inoculation assays (*S. riobrave* inoculated together with T. *semipenetrans*), a reduction of the number of females of T. semipenetrans parasitizing the citrus roots was observed when compared to the controls (Fig.1). This was valid for both the low and high *S .riobrave*-inoculum concentrations. When *S. riobrave* was added after the establishment of *T. semipenetrans*, the high *S. riobrave* dosis did better when compared to the low concentration dosis (Fig. 2).

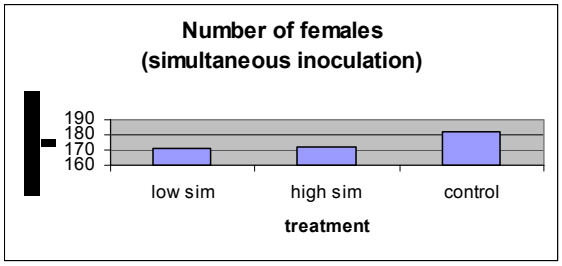

Figure 1

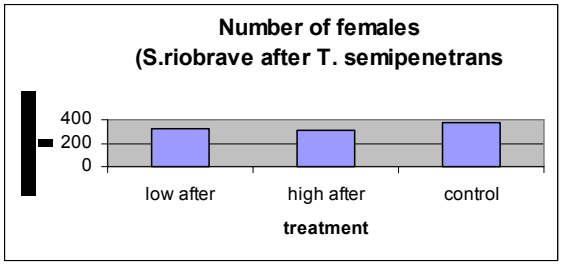

Figure 2

A reduction in the number of egg masses was also observed for both the simultaneous and after-*T. semipentrans*-establishment inoculation times. In both situations, the higher *S. riobrave* inoculum dosage performed better in reducing the number of egg masses of the citrus nematode (Figs. 3, 4)

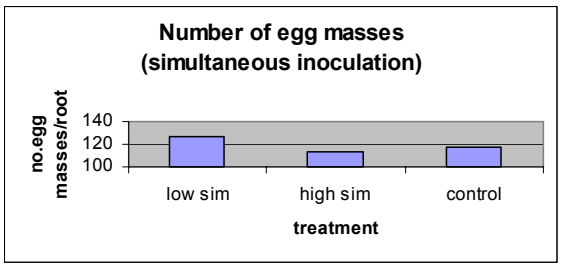

Figure 3

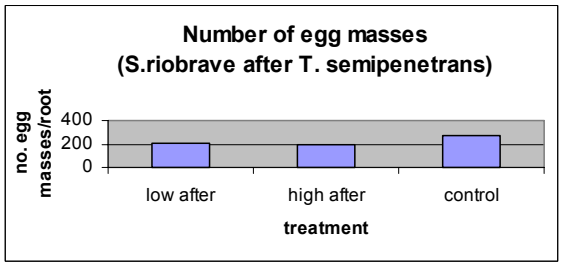

Figure 4

A reduction in number of infective juveniles (J2) penetrating the citrus roots was also observed both for the high and low inoculum *S. riobrave* concentrations,regardless if T. *semipenetrans* nematodes were exposed to *S. riobrave* before or after their establishment in the citrus roots (Figs.5, 6).

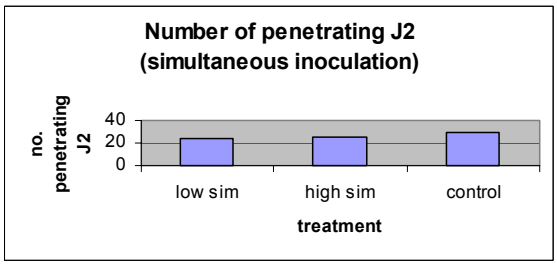

Figure 5

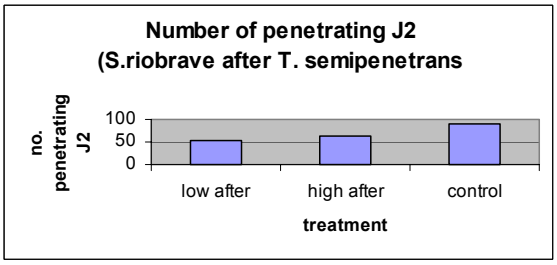

Figure 6

Fresh root weight was also measured to account for the impact of *S. riobrave* on *T. semipenetrans* infection in the citrus seedlings. A significant reduction of the root weight was only observed when a high inoculum of *S. riobrave* was applied at the same time *T. semipenetrans* was inoculated. For all other cases, no significant differences were observed (Figs.7, 8)

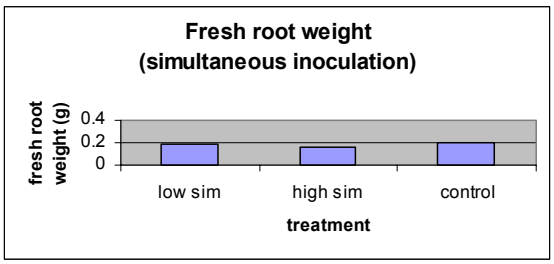

Figure 7

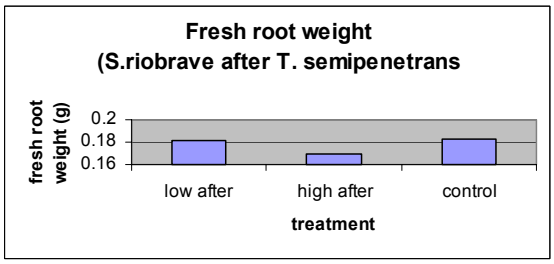

Figure 8

Conclusions

Our results indicate that, unlike what Nigh et al (1997) demonstrated there seems to be an interaction between the citrus nematode *T. semipenetran*s and the entomopathogenic nematode *S. riobrave*. However, the nature of this interaction cannot be understood by the experiments we conducted. It would be valuable to conduct further experiments to expand these studies trying to optimize the doses rate of *S. riobrave* and

also evaluate different application times (i.e. considering different intervals prior and after establishment of *T. semipenetrans).*

Finally, we consider our results are very encouraging and with the growing concerns about ecological ramifications of inundative and inoculative releases of biological control agents, the need for the understanding of these sort of interactions is necessary. Moreover, with the eminent removal of many traditional chemical pesticides from the market, the study of nematicidal properties of entomopathogenic nematodes against plant-parasitic nematodes might be a useful tool for the control of these plant parasites.